

Phospholipids and wheat gluten blends: interaction and kinetics[☆]

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Received 4 November 2003; revised 19 August 2004; accepted 22 August 2004

Abstract

A model system comprising of lysophosphatidylcholine (LPC) and isolated gluten were used help understand the positive effect of PL on bread-loaf volume. The kinetics of the effect of gluten on the thermal properties of LPC were determined using DSC. Blends of PL and 3, 6, and 10% gluten were heated from 0 to 70 °C at rates between 3 and 19 °C/min and cooled to 0 °C. The onset and peak temperatures and ΔH were recorded. The peak temperature was used to calculate the activation energy (E_a) and Z value. The transition for pure LPC vesicle formation was detectable by DSC in the presence of gluten. Gluten increased the activation energy of LPC during vesicle formation and disruption. The increase in gluten content from 3 to 6% and then to 10% had a slight effect on the activation energy value of LPC during vesicle disruption, whereas during formation a steady increase was noticed with higher gluten additions. Overall, the ΔH of the blends showed a decrease at higher heating rate. The change in the PL activation energy in the presence of gluten is indicative of a form of interaction.

Published by Elsevier Ltd.

Keywords: Phospholipids; Wheat gluten; Kinetics; Activation energy; Poly-glutamic acid; Differential scanning calorimetry

1. Introduction

Although lipids are present in small quantities in wheat, they have a significant effect on the final texture of food products. Available data indicate that polar lipids i.e. phospholipids and glycolipids interact primarily with wheat gluten protein (Pomeranz, 1980). This is significant because the protein network is the backbone of wheat flour dough mixing properties and gas retention (Chung and Pomeranz, 1977; Chung et al., 1978; Mecham, 1971; Morrison, 1978; Pomeranz, 1971, 1980). Wheat lipids are

divided into two groups: non-polar and polar. Morrison et al. (1975) reported that non-polar lipids are dominated by triglycerides and they originate from the embryo and liposomes in the endosperm. Polar lipids are found mainly in cell membranes and consist mostly of phospholipids such as lysophosphatidylcholine (LPC). At high concentration polar lipids may form a bilayer in which the polar ends face the aqueous environment. Bilayers of wheat phospholipids are capable of interacting with wheat proteins through both their polar and non-polar ends. Phosphorous NMR of wheat gluten showed that phospholipids are organized in a lamellar liquid crystalline phase. Interactions between gluten and phospholipids are sensitive to temperature and mechanical work. The interruption of these interactions results in the expulsion of the phospholipids from the protein network into the water phase during extensive mechanical work or heating and cooling. The interactions between phospholipids and gluten, as shown by NMR, are different from that occurring in cell membranes (Didier et al., 1987). Some free lipids become bound after flour is wetted and formed into dough.

Abbreviations: DSC, differential scanning calorimetry; FTIR, Fourier transform infrared (FTIR) spectroscopy; GLM, general linear model; LPC, L- α -lysophosphatidylcholine; PG, poly-glutamic acid.

[☆]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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The thermal properties and the kinetics of phospholipid micelle disruption and formation can be determined by DSC. Ozawa (1970) demonstrated that non-isothermal DSC data can be used to determine activation energy, with the assumption that peak temperature determined by DSC is also the temperature of the maximum reaction rate. Reaction rate for a solid A to produce a solid product B can be expressed as follows

$$\frac{dx}{dt} = Z e^{-E_a/RT} (1-x)^n \quad (1)$$

where x , fraction reacted; Z , pre-exponential factor; n , reaction order, E_a , activation energy; T , absolute temperature and R , universal gas constant. If the temperature of a first-order reaction ($n=1$) is raised at constant rate, β and $T=T_p$ (T_p is DSC peak temperature), Eq. (2), derived according to Ojeda et al. (2000), makes it possible to determine the activation energy of the interaction using DSC data

$$\ln\left(\frac{\beta}{T_p^2}\right) = \ln\left(\frac{RZ}{E_a}\right) - \left[\left(\frac{E_a}{R}\right)\left(\frac{1}{T_p}\right)\right] \quad (2)$$

where β , constant rate of temperature rise; T_p , peak temperature; R , universal gas constant; Z , pre-exponential factor; and E_a , activation energy. The natural logarithm transformation is used to linearize the equation.

From Eq. (2), the plot of $-\ln(\beta/T_p^2)$ against $1/T_p$ will represent a straight line with a slope $=R/E_a$, from which the activation energy (E_a) value can be calculated.

The objective of this work was to estimate the change in kinetics of the LPC interaction with wheat gluten, during the heating of a water suspension of the mixture using DSC and FTIR spectroscopy. Of the two components, the physico-chemical changes in LPC were detectable by DSC, whereas gluten showed no transition at concentrations used in this work.

2. Materials and methods

2.1. Materials

Egg yolk L- α -lysophosphatidylcholine (LPC), gluten, and poly-glutamic acid (PG) (degree of polymerization 180) were from Sigma Chemical Company (St Louis, MO). Purified vital gluten was prepared by heating gluten slurry to 95 °C and holding for 10 min to gelatinize the starch, centrifuging and freeze-drying the pellet. To ensure that the isolation process had not altered the gluten structures, gluten purchased from Sigma, native gluten from Hard Red Spring (HRS) wheat flour, commercially available vital gluten, and purified vital gluten samples were compared using SDS-PAGE (Mohamed and Rayas-Duarte, 2003). The gluten was used without further purification. A 40% LPC

water suspension was prepared with 3, 6, or 10% gluten, based on the LPC weight, or with PG at 10%.

2.2. DSC analysis

For DSC analysis 5 mg of mixtures were placed in aluminum DSC pans and analyzed using a TA 2920 DSC (TA Instruments Thermal Analysis and Rheology, New Castle, Delaware). Heating was in the range -5 to 80 °C with a heating rate between 3 and 19 °C/min with an increase of 2 °C/min. At -5 and 80 °C a 1-min iso-track was used. Samples were subjected to heating and cooling cycles to test the ability of LPC to reorganize and show transitions after cycling. A total of five heating and cooling cycles were run. The second cycle was used for the calculation of kinetics.

2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR Spectroscopy was performed on model systems consisting of LPC and PG. A 50:50 blend of LPC and PG was dispersed in distilled water at 40% concentration and held at 30 °C for 16 h to allow the polypeptide to interact completely with the phospholipid multilayers. The dispersion was freeze-dried and pulverized under liquid nitrogen (-196 °C) to give a fine powder that was used to obtain the test spectrum. Pure LPC and PG were each treated separately, as above, to prepare a control spectrum as the sum of the two pure component spectra.

Samples of the test PG-LPC blend and the pure PG and LPC were pulverized with KBr and pressed into transparent disks for analysis by FTIR spectrometry. A test sample (2.00 mg) was pulverized at liquid nitrogen temperature in a sealed stainless steel vial containing a stainless steel ball bearing for 15 s on a Wig-L-Bug amalgamator (Crescent Dental Manufacturing, Lyons, IL). The vial was allowed to warm to room temperature before KBr (698 mg) was added. The KBr/sample mixture was pulverized at liquid nitrogen temperature in the same vial with the same ball bearing on the amalgamator for 10 s. The vial was again allowed to warm to room temperature before 300 mg of the KBr/sample mixture was transferred to a KBr die (Perkin-Elmer Corp. Norwalk, CT) and pressed under vacuum at 110 MPa on a laboratory press (Fred S. Carver, Menominee Falls, WI).

FTIR spectra were measured on an FTS 6000 spectrometer (Digilab, Cambridge, CT) equipped with a DTGS detector. Absorbance spectra were acquired at 4 cm^{-1} resolution and signal-averaged over 32 scans. Interferograms were Fourier transformed using triangular apodization for optimum linear response. Spectra were baseline corrected, truncated to span only the methylene and methyl band range ($3000\text{--}2800\text{ cm}^{-1}$), and scaled to adjust for small differences in sample weights.

2.4. Experimental design and statistical analysis

A Completely Random Design was used in a General Linear Model (GLM) approach for modeling LPC vesicle disruption (melting) and formation (crystallization) kinetics of LPC with gluten or poly-glutamic acid additions. The first order reaction equation was derived from the Ozawa model and reported by Ojeda et al. (2000) for determining rate constants and activation energies from differential thermal analysis and is presented as Eq. (2).

Rewriting Eq. (2) in standard simple linear regression form, $Y = mX + b$, where m , slope and b , intercept, we obtain equation

$$\left(\frac{1}{T_p}\right) = \left(\frac{R}{E_a}\right) \left[-\ln\left(\frac{\beta}{T_p^2}\right)\right] + \left(\frac{R}{E_a}\right) \ln\left(\frac{RZ}{E_a}\right) \quad (3)$$

where $Y = (1/T_p)$, $X = -\ln(\beta/T_p^2)$, slope $m = (R/E_a)$, and intercept $b = (R/E_a) \ln(RZ/E_a)$.

Five separate regression equations were obtained consisting of phospholipid treatments with 0, 3, 6, and 10% added gluten, and phospholipids plus 10% added poly-glutamic acid. The form of the dependent Y -variable and the form of the independent X -variable are as defined in Eq. (3), for both melting and crystallization phases of the thermal reaction (SAS Institute, 1991b). Two separate GLM F -tests for full and reduced models were used to test for differences between the phospholipid treatment equations for the melting and crystallization phases of the experiment (Neter et al., 1990). When a significant GLM F -test was obtained, indicating that at least one of the equations was different from the rest, distance metrics were used as a multiple comparison test for determining which treatment equations were different from the others (Palmquist, 1993).

Z values were calculated and compared for the melting and crystallization phases by using the slope m and intercept b values from Eq. (3) to obtain

$$Z = \frac{1}{m} e^{(b/m)} \quad (4)$$

PROC REG from SAS PC Windows Version 8.2 was the statistical software used for most of the analyses (SAS Institute, 1991a).

3. Results and discussion

3.1. Comparative

SDS-PAGE analysis of the glutes showed that gluten purchased from Sigma was similar in electrophoretic profile to proteins from Hard Red Spring wheat flour, commercially available vital gluten, and purified vital gluten samples (Fig. 1). The DSC data showed that LPC has an endothermic (heating) and exothermic (cooling) peak transitions at 55 and 48 °C, respectively. The DSC transition profiles of LPC

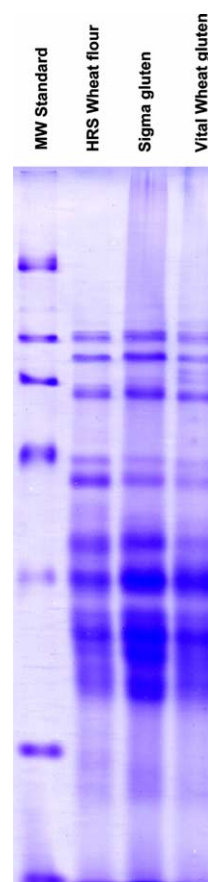


Fig. 1. Reduced SDS-PAGE of Hard Red Spring wheat (HRS), vital gluten, and purified vital gluten.

mixed with gluten showed a gradual decrease in the size of both transitions with increase in the gluten content (Fig. 2). The NMR data reported by Didier et al. (1987) indicated that phospholipids are immobilized in the gluten matrix

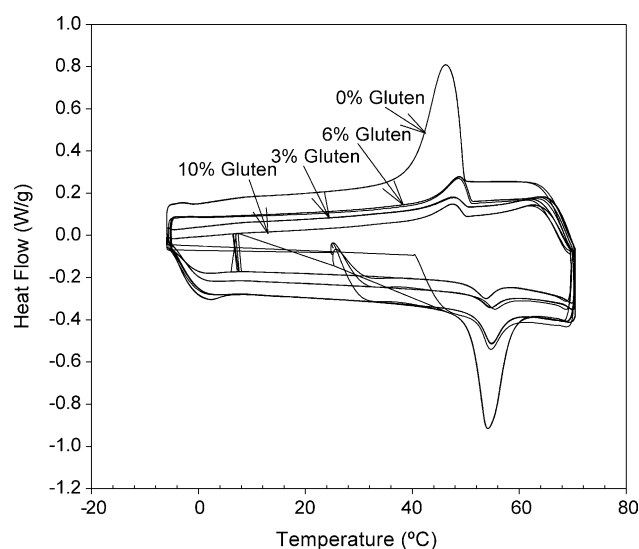


Fig. 2. LPC profiles of mixtures with 3, 6, and 10% gluten.

and become mobile as the system is cooled or overmixed. The DSC data reported here showed that the effect of gluten on the LPC is not reversed by cooling. The LPC–protein system analyzed by DCS and FTIR was prepared differently from that analyzed by NMR. In the present work the LPC–protein system had a limited amount of gluten, unlike that analyzed by NMR, where the phospholipid content was limited. The reduction in the ΔH of LPC transition, at higher gluten content, is indicative of some sort of penetration of gluten into the LPC bilayer. The low ΔH indicates molecular disorder of bilayers as the amount of gluten increased. Papahajopoulos et al. (1975) reported binding of ribonuclease to di-palmitoylphosphatidylglycerol (a charged phospholipid) as shown by higher peak temperature and ΔH , but ribonuclease had no effect on the thermal properties of LPC (a neutral phospholipid). The same authors reported that gramicidin, a hydrophobic protein, reduced the ΔH of both types of phospholipids but had only small effect on peak temperature. The data reported here showed reduced ΔH values and small changes in the peak temperature. This is consistent with the hydrophobic nature of wheat gluten. The lower ΔH values unaccompanied by a change in the peak temperature indicate some kind of penetration of gluten into the LPC bilayer but not enough to compromise the LPC vesicle structure. The evidence for this is shown clearly by the ability of LPC to form vesicles as the system cooled. LPC could be immobilized by the protein or strongly bound to it (Didier et al., 1987). A substantial change in the peak temperature is definitive evidence of disturbance of the closely packed LPC vesicle structure. Gluten protein has a substantial effect on LPC peak temperature when compared to the heating and cooling cycles. In the presence of different levels of gluten the peak temperature of LPC was 4–6 °C lower during the cooling cycle compared to heating (Fig. 2). Perhaps, lower molecular mobility during cooling limited the contact between LPC and gluten and thus allowed LPC vesicles to rearrange at the lower temperature.

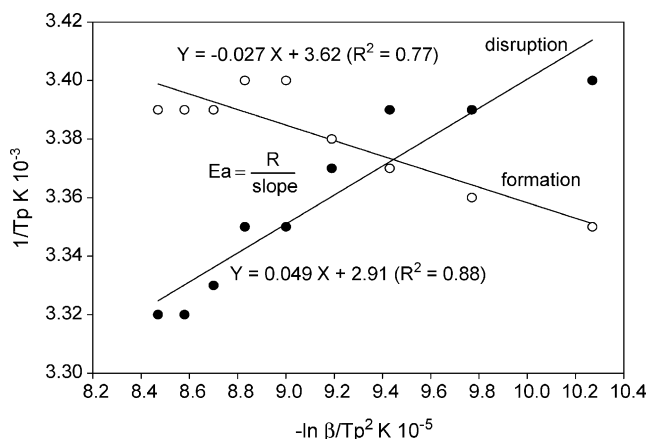


Fig. 3. Pure LPC vesicles disruption and formation.

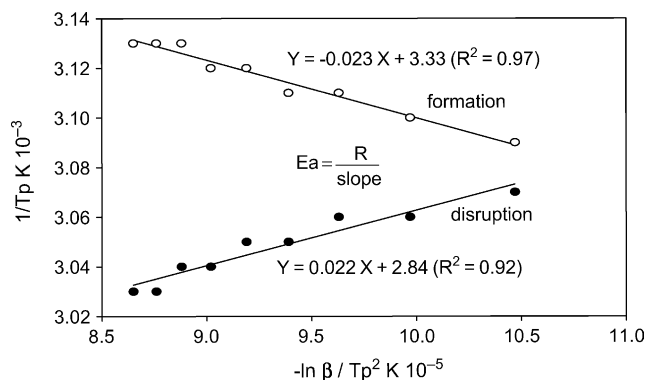


Fig. 4. Disruption and formation of LPC vesicles in 3% gluten.

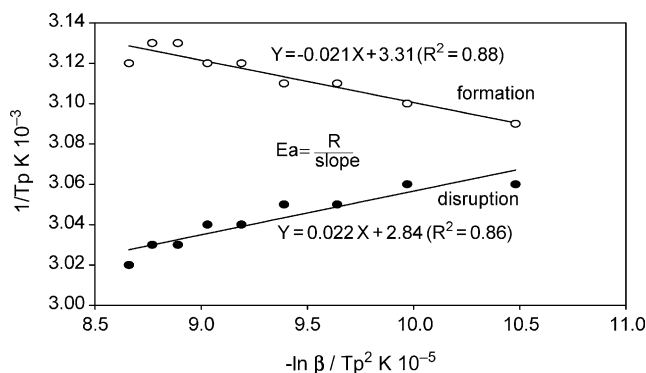


Fig. 5. Disruption and formation of LPC vesicles in 6% gluten.

The best-fit regression equations used for predicting $Y_1 = (1/T_p)$ of melting and $Y_2 = (1/T_p)$ of crystallization for $X = -\ln(\beta/T_p^2)$ as defined in Eq. (3), are included in Figs. 3–6.

The DSC kinetics of LPC vesicle disruption and formation was calculated according to Ozawa (1970). The pure LPC kinetics are shown in Table 1 and Fig. 3. Table 1 provides an example of how the data for the LPC/gluten blends were calculated. The calculated activation energy (E_a) of LPC vesicle disruption and formation was 167.9 and 307.9 kJ/mol, respectively (Fig. 7). Higher LPC E_a values during cooling, in a hydrophilic environment, point to slower vesicle formation compared to E_a during heating

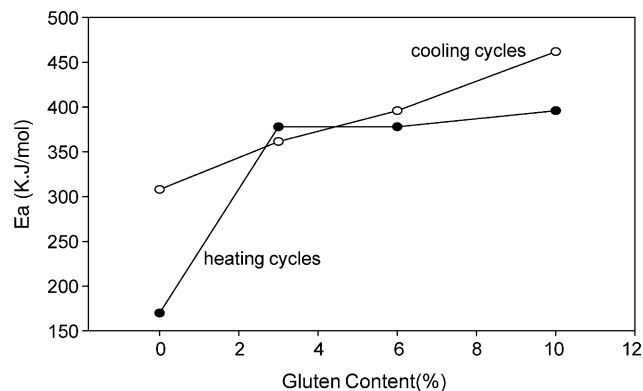


Fig. 6. Disruption and formation of LPC vesicles in 10% gluten.

Table 1
Kinetics relationship between heating or cooling rates and peak temperature for pure phospholipids according to Ozawa (1970)

| Heating rate (β) | Peak T_p ($^{\circ}\text{C}$) | Peak T_p (K) | $(1/T_p) \times 10^{-3}$ | $(\beta/T_p^2) \times 10^{-6}$ | $(-\ln(\beta/T_p^2)) \times 10^{-5}$ |
|---|-----------------------------------|----------------|--------------------------|--------------------------------|--------------------------------------|
| <i>Micelles melting (heating)</i> | | | | | |
| 3 | 52.4 | 325.6 | 3.07 | 2.83 | 10.47 |
| 5 | 53.3 | 326.4 | 3.06 | 4.69 | 9.97 |
| 7 | 53.5 | 326.6 | 3.06 | 6.56 | 9.63 |
| 9 | 54.0 | 327.1 | 3.06 | 8.41 | 9.38 |
| 11 | 54.4 | 327.6 | 3.05 | 10.25 | 9.19 |
| 13 | 54.9 | 328.0 | 3.05 | 12.09 | 9.02 |
| 15 | 55.3 | 328.5 | 3.04 | 13.90 | 8.88 |
| 17 | 55.8 | 328.9 | 3.04 | 15.71 | 8.76 |
| 19 | 56.3 | 329.5 | 3.04 | 17.50 | 8.65 |
| <i>Micelles crystallization (cooling)</i> | | | | | |
| 3 | 48.7 | 321.9 | 3.11 | 2.90 | 10.45 |
| 5 | 47.9 | 321.2 | 3.11 | 4.85 | 9.93 |
| 7 | 46.9 | 320.1 | 3.12 | 6.83 | 9.59 |
| 9 | 46.2 | 319.3 | 3.13 | 8.83 | 9.34 |
| 11 | 45.4 | 318.5 | 3.14 | 10.84 | 9.13 |
| 13 | 44.6 | 317.8 | 3.15 | 12.87 | 8.96 |
| 15 | 44.3 | 317.5 | 3.15 | 14.88 | 8.81 |
| 17 | 44.3 | 317.4 | 3.15 | 16.87 | 8.69 |
| 19 | 44.3 | 317.4 | 3.15 | 18.86 | 8.58 |

where vesicle disruption was faster as indicated by lower E_a values. In the presence of 3% wheat gluten, the E_a has increased by 200 kJ/mol during vesicle disruption, whereas only 60 kJ/mol increase was observed during the vesicle formation cycle (cooling) (Fig. 4). The addition of 6% gluten increased E_a by 210 kJ/mol during heating, while the cooling cycle increased the E_a value by 89 kJ/mol (Fig. 5). Finally, the 10% gluten increased the E_a of vesicle disruption by 229 kJ/mol and the vesicle formation by 155 kJ/mol (Fig. 6). The overall effect of gluten addition on the LPC vesicle disruption and formation E_a values is presented in Fig. 6. It is obvious from Fig. 6 that a large increase in E_a of vesicle disruption occurred with the addition of 3% gluten, but at 6 and 10% there was a limited effect on the increase in E_a . During the vesicle formation step, it appears that the overall increase in E_a values is lower than that for vesicle disruption, and its magnitude is related

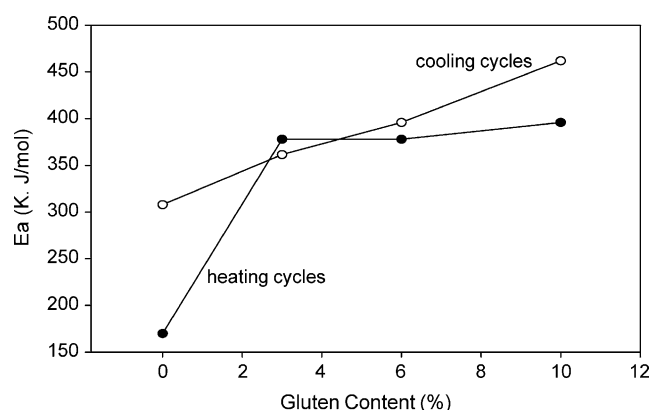


Fig. 7. Activation energy of LPC blends with 3, 6, and 10% gluten.

Table 2
Results of use of distance metric for determining treatment equation differences

| Phospholipids treatment (%) | Phase | |
|-----------------------------|----------------|-----------------|
| | Melting | Crystallization |
| 0 Gluten | a ^a | a |
| 3 Gluten | b | bc |
| 6 Gluten | b | b |
| 10 Gluten | b | b |
| 10 Poly-glutamic acid | b | C |

Phospholipids treatments followed by the same letter(s) within a column indicates no significant differences between equations based on distance metric calculations.

to the amount of gluten added. In the presence of poly-glutamic acid, E_a value increased by 350 kJ/mol during vesicle disruption when compared to the control and reduced by 30 kJ/mol during formation. This was the only situation where E_a of the control was reduced. The reduction in the E_a compared to the E_a of pure LPC could mean that the poly-glutamic acid peptide changed structure during the heating cycle in a way similar to poly-lysine as reported by Hammes and Schullery (1970). The change in the structure seemed to facilitate faster vesicle formation.

All equations were statistically significant, as were all slope and interaction coefficients for the models ($p < 0.001$), indicating true, non-zero contributions to the prediction equations.

The GLM F -tests for determining melting phase equation differences (Y_1) uses a full model (separate regression equation for each phospholipid treatment) and reduced model (one equation for all phospholipid treatments) analysis with test statistics signifying at least one of the regression lines is not equal to the others at the 1% α level. The phospholipid treatments have different ($1/T_p$) responses from one another in the melting phase. The GLM F -tests for determining crystallization phase equation differences (Y_2) resulted in test statistics signifying that at least one of the regression lines is not equal to the others at the 1% α level. The phospholipid treatments have different ($1/T_p$) responses from one another during the crystallization phase. The difference between the crystallization phase relative to the melting phase is due to the lower peak temperature during crystallization (Table 2). The Z value (pre-exponential factor) during the LPC vesicle disruption increased with

Table 3
Calculated Z value using Eq. 4 with slope and intercept values from the above regression models

| Phospholipids treatments (%) | Phase | |
|------------------------------|------------------------|--------------------------|
| | Melting | Crystallization |
| 0 Gluten | 6.354×10^{26} | -1.625×10^{-58} |
| 3 Gluten | 1.086×10^{57} | -2.574×10^{-61} |
| 6 Gluten | 3.796×10^{58} | -1.238×10^{-67} |
| 10 Gluten | 1.235×10^{60} | -2.308×10^{-78} |
| 10 Poly-glutamic acid | 1.000×10^{80} | -2.714×10^{-47} |

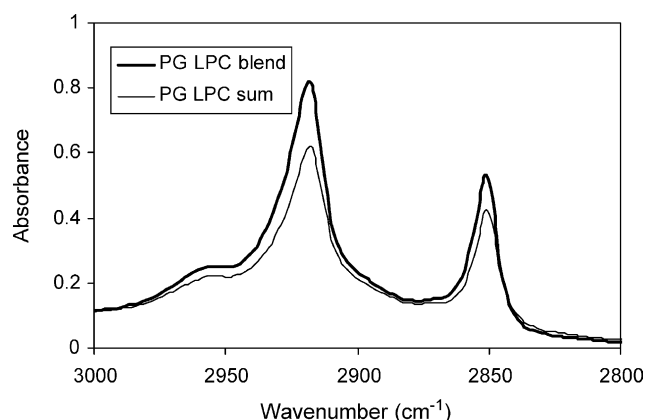


Fig. 8. Comparison of FTIR spectrum of PG–LPC blend with the Beer's law addition spectrum.

the increase in gluten content (Table 3). The Z value decreased during vesicle reformation

The infrared technique was also used here to investigate the effect of PG on the conformation of LPC in aqueous dispersion. It is well known that the infrared spectra of proteins and lipids are sensitive to their molecular conformation (Painter and Koenig, 1976) and the interactions of polypeptides with phospholipids have been extensively studied using FTIR Raman spectroscopy (Bertoluzza et al., 1983; Carrier and Pézolet, 1984; Pézolet et al., 1982).

According to Koenig (1983), molecular interactions between polymeric materials in a blend can be detected by showing that a significant difference exists between the spectrum of the blend and the sum of the spectra of the pure components. This is the conventional Beer's law spectral addition technique, which, despite its drawbacks (Gordon et al., 2004), is acceptable for two-component systems. Applied here, and plotted for PG and LPC in Fig. 8, the spectral addition technique showed a significant difference between the spectrum of the blend and the sum of the spectra of the pure PG and LPC components. The spectra were normalized at the nearest absorbance minimum (3004 cm^{-1}) and plotted over the $3000\text{--}2800\text{ cm}^{-1}$ range to make the comparison based on the absorbance intensity ratio, $I_R = I_{2920\text{ cm}^{-1}}/I_{2850\text{ cm}^{-1}}$, defined by Verma and Wallach (1978) and Wallach et al. (1979). As described by Bertoluzza et al. (1983) this intensity ratio is related to the mobility of terminal methyl groups involved in conformation changes in phospholipids caused by interaction with protein. As shown in Fig. 8, the absorbance intensity ratio for the sum of PG and LPC is $I_R = 1.46$ while the ratio for the PG–LPC blend is $I_R = 1.55$. This result is qualitatively similar to the behavior observed by Bertoluzza et al. (1983), now using a non obsolete dispersive Raman spectrometer. The state-of-the-art FTIR spectroscopic result confirms quantitatively that the conformation of the lipid bilayer is changed by penetration of poly-glutamic acid into the lysophosphatidylcholine membrane. Thus, FTIR analysis of this model system provides good evidence that

phospholipid–gluten surface interactions most likely occur at their interface in aqueous systems and play an important role in dough mixing and determining bread-loaf volume. The interaction between LPC and gluten facilitates gluten's ability to form the network needed for effective dough formation. Since LPC has the capability to interact with gluten via hydrophobic interaction and with the continuous aqueous component of the dough, it will influence the effectiveness of gluten network formation. The interaction between LPC and gluten may physically help gluten to extend to cover more dough surface and thus increases gas retention.

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